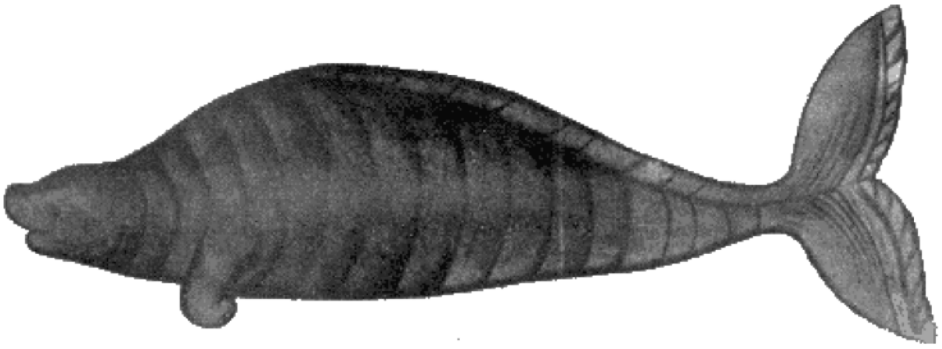
Climate change: a view through the prism of Steller's sea cow extinction

BRIEF OVERVIEW OF 2011 FIELD WORK

Presented by
Alexander Burdin,
SFOS UAF, Kamchatka Branch of Pacific Institute of Geography RAS, Petropavlovsk-Kamchatsky, Russia

Brooke Potgieter and Gregory O'Corry-Crowe
Harbor Branch Oceanographic Institute, Florida Atlantic University, Fort Pierce, Florida



Field work

The main goal of the 2011 field work remained the same as with the first field season of our project: to collect Steller's sea cow bones on the beaches of Bering Island, take photos and video and work with Nikolskoe village community, explaining the importance of collecting the valuable Steller's sea cow remains.

As in our previous season only one of the collaborators, Alexander Burdin, was able to conduct fieldwork on Bering Island in 2011. Students from Russian universities were also involved in the bone collection process and some people from Nikoslkoe donated a few Steller's sea cow bones.

In summer 2012 we rented the same 4-wheeler in Petropavlovsk-Kamchatsky, and it was shipped by cargo boat with gas and other supplies to Bering Island as the main transport for collection of the bones. A new boat and outboard motor were purchased in Petropavlovsk-Kamchatsky, and shipped to Nikolskoe. We used this boat to visit distant locations on Bering Island that we could not reach by the 4-wheeler.

For some logistic and safety reasons we postponed our trip to Medny Island, but we did get a few samples from the bones collected on Medny Island that provided for our research needs. Because Steller's sea cows inhabited both Bering and Medny Islands, we were expecting that exchange between these two populations was very limited; thus, it is extremely important to compare samples from both islands.

In total in 2011, Alexander Burdin spent two month in the field. As usual, weather conditions in the Commander Islands in the summer 2011 were not very supportive. There were a lot of rainy, stormy days when it was impossible to operate the 4-wheeler, and that reduced our efforts. Based on Nikolskoe village, 12 long trips were made by the 4- wheeler to the east coast, travelling up to Tolsty Cape (about 90 km), to Sarannoe Lake, to Northwest Cape, and to Poludennaya Bay on the west coast of Bering Island. The southern part of Bering Island's west coast was reached by inflatable boat.

We realize that increasing our efforts for bone collection after a short period of time has limitations, and may not bring desirable results, because all noticeable bones on the beaches are usually collected in a one trip. It is best to wait for further collection until a storm will move sand or gravel, or to try to visit previously unchecked locations.

A Steller's sea cow rib on the sandy beach of Commander Bay on the east coast of Bering Island. This is the bay where in November 1741 boat the St. Peter was crushed, and Vitus Bering and his 17 companions soon died and were buried. The rest of the team spent a severe winter camping in this bay. No one knows, but maybe this is a rib of a sea cow killed by the starving survivors of the Vitus Bering expedition.

Results

In total, in the 2011 field season, 44 Steller's sea cow ribs, one scapula and three vertebrae were collected on our trips along the Bering Island beaches, mainly on the east coast, and 12 more ribs were discovered at Nikolskoe village in the Commander Islands Nature Reserve storage that had been acquired from local citizens. These bones were collected for different goals; for example a scientist from Commander Islands Nature Reserve made an excavation of an almost full Steller's sea cow skeleton, and all the bones belonged to a single animal. But mostly people collected bones as a potential source for carving and souvenir production. In spite of illegal trade of Steller's sea cow bones, it is possible to find carvings in the souvenor stores in Kamchatka peninsula and even in other Russian Far East towns. We suggest it was very important to sample these bones, for the following reasons:

1. Usually collectors select bones of good quality: dense, without damages, and with a good upper layer.
2. It seems to us, that ribs and bones in such good condition are probably the most recent, and present the last generations of Steller's sea cows prior to human contact and soon after that. The more of these "recent" bones we can analyze, the more accurate we can estimate genetic variation of this species.

In 2011, 40 more Steller's sea cow bone samples were provided for genetic analysis to Dr. O'Corry-Crowe, and the development samples collected in 2010 are now in progress. This laboratory analysis was initiated because the National Park Service agreed to use some of the grant funds to buy supplies for genetic research.

Laboratory analysis

Methods

Sea cow bone samples were collected on the Commander Islands by Alexander Burdin and colleagues. Upon discovery of each fragment or set of bones, a detailed description of the field site (including GPS co-ordinates) was made prior to excavation and sample collection and labeling followed strict guidelines to ensure integrity and limit contamination. Using a sterile lab and equipment, Burdin drilled up to 5gr of bone powder from an EtOH swabbed section of bone, discarding 5mm of surface material to limit environmental contamination of sea cow DNA. (*See* Report on Phase 1A sample collection for further details).

Total DNA was extracted from 11 bone powder samples using a silica-based aDNA approach (Pääbo, 1989; Hofreiter et al. 2004; Rohland and Hofreiter 2007). A modified version of the extraction method of Höss and Pääbo (1993), as described in Hofreiter et al. (2004) was used on all samples. The extractions were done in small batches with two extraction controls in each batch. The powder was added to 3.3ml of lysis buffer containing 5% N-lauroylsarcosine, 8mg/ml Dithiothreitol (DTT), 1% Polyvinyl polypyrrolidone (PVP), 0.45 M EDTA pH 8.0 , and 0.25mg/ml Proteinase K, and incubated at 37ºC for 12-16 hrs rotating continuously. After centrifugation for 2 minutes at 2000 rpm, the supernatant was added to 11.3ml of extraction buffer containing 5.5 M Guanidine thiocyanate (GuSCN), 0.05 M Tris pH 8.0, 0.025 M NaCl, and 20µl of a silica suspension prepared similar to Boom et al. (1990). The DNA bound to the silica was recovered through multiple washes in an ethanol solution, and elution in 100ul of Tris EDTA buffer. This method was previously used on one Steller's sea cow sample (G. O'Corry-Crowe, M. Meyer and A. Frey, unpubl.) and provided sufficient quantity and quality of total DNA from 9 samples to conduct mtDNA PCR and sequencing.

Amplification of the target DNA was also performed within the Ancient DNA laboratory in a separate clean-room with more stringent air quality rating (Class 1000). The samples were initially spun to pellet any remaining silica. Target DNA was amplified using the polymerase chain reaction in 50µl reactions containing 2-8µl of an unknown amount of DNA, 16mM $(NH_4)_2SO_4$, 67mM Tris-HCl pH 8.8, 0.01% Tween-20, 150µM of each dNTP, 0.3µM of each primer, and 2.5 units of Biolase™ DNA Taq Polymerase. Reactions were run on an Applied Biosystems 2700/2720 Thermal Cycler. The profile consisted of an initial denaturation for 5:00 min at 94ºC, then 0:30 min denaturation at 94ºC, 0:45 min annealing at 46ºC, and 1:05 min extension at 72ºC for 35 cycles followed by a final extension period of 10 min at 72ºC. Using multiple pairs of primers that amplify overlapping segments of the mtDNA genome, we attempted to amplify mtDNA fragments of between 250 and 700bp. Somewhat surprisingly, the longest fragment, spanning most of the threonine tRNA gene, the proline tRNA gene and much of the control region, amplified the most consistently and strongest. Using the same primers as used in the original amplification we sequenced both strands of the amplicon for samples

that yielded strong, clean PCR fragments.

Results

Total DNA was extracted from 11 bone powder samples of Steller's sea cow collected from sea cow bones at six sites on Bering Island between 1994 and 2010. A roughly 700bp fragment of mtDNA was successfully amplified from nine samples. Subsequent sequence analysis yielded high quality light and heavy strand sequence for 581bp of this fragment in seven individuals (Figure 1). An eighth sample yielded good quality sequence from a somewhat shorter read length (Table 1).

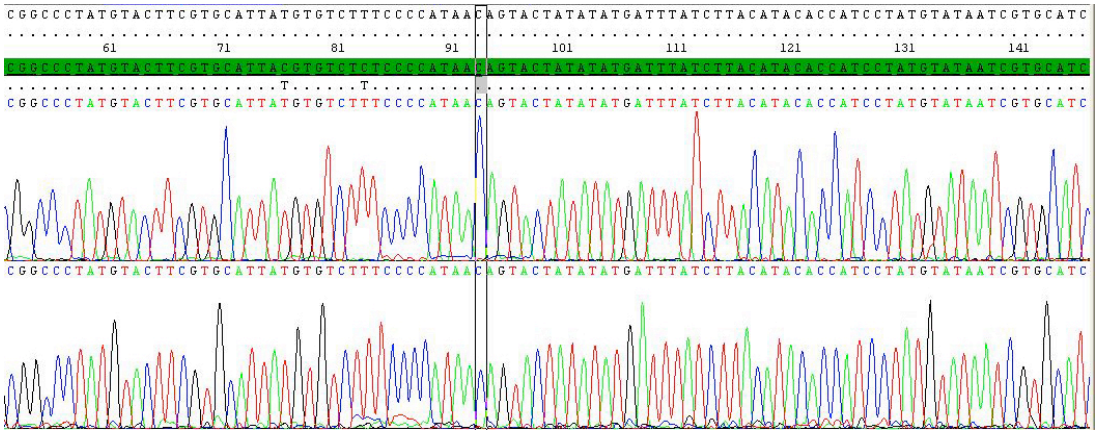


Figure 1. *An electropherogram of Steller's sea cow (Hydrodamalis gigas) mtDNA control region sequence from DNA extracted from sea cow bones collected on Bering Island, the larger of the two main Commander Islands, Russia.*

Six variable sites defining three unique mtDNA haplotypes were documented among the seven samples successfully sequenced for the entire 581bp (Figure 2). Two of the haplotypes were found in a single sample, the third was documented in the remaining five samples. While there is a possibility that some of the bone samples with the same haplotype may have originated from the same individual, the sample and haplotype distribution indicate a minimum of x samples.

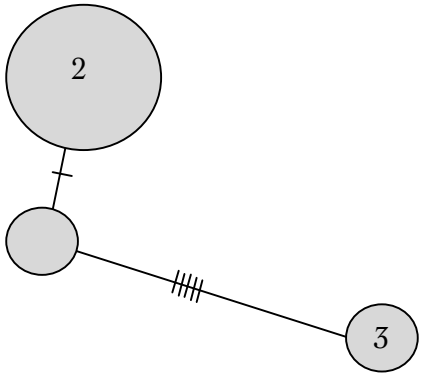


Figure 2. Minimum spanning network of Steller's sea cow mtDNA control region haplotypes.

HBOI ID	Field ID	Bone Type	Collection Location	Date Collected	DNA Successfully Extracted	DNA Amplified by PCR	DNA Succesfully Sequenced	Haplotype
HA36	1	Rib	Bering Island – Drovenskaya bay	07/25/94	x	x	Y	1
HA37	2	Rib	Bering Island – Drovenskaya bay	07/26/94	x	x	Y	na
HA38	3	Rib	Bering Island – Peredovaya bay	07/27/94	x	x	Y	2
HA39	4	Rib	Bering Island – NW cape	03/03/95	x	x	Y	3
HA40	5	Rib	Bering Island – Manati cape	05/30/95	x	x	N	na
HA41	6	Rib	Bering Island – Bobrovaya bay	07/24/95	x	x	Y	could not call, seq too short
HA42	7	Rib	Bering Island – Poludennaya bay	08/30/09	x	x	Y	2
HA43	8	Rib	Bering Island – Poludennaya bay	08/31/09	x	x	Y	2
HA44	9	Rib	Bering Island – ND	09/04/09	x	x	N	na
HA45	10	Rib	Bering Island – ND	09/04/09	x	x	Y	2
HA46	11	Rib	Bering Island – ND	09/04/09	x	x	Y	2

Table 1. Summary of Steller's sea cow bone samples collected on Bering Island and analyzed as part of this pilot aDNA study. Success in each process is denoted by an 'x'.

Discussion

This pilot study successfully established an efficient lab method for studying aDNA from Steller's sea cow, and has provided the first data on genetic diversity within this extinct sirenian. A concern with such aDNA studies is that the quality and quantity of DNA extracted from the material will be low, thus affecting PCR success and sequencing quality. Few aDNA studies of large sample sets have been conducted for these reasons, limiting the application of this technology in intra-specific studies where sample sizes and read lengths have to be quite large. The quality and length of DNA sequence reads for Steller's sea cow mtDNA in the current study are exceptional, even for contemporary tissue samples, and augur well for high throughput analyses of bone samples necessary for quantitative genetic analysis. We are confident that we can expand the scope of this study to include more samples across a longer time frame, and more genetic markers, including genes associated with individual fitness and population viability.

Understanding the genetic aspects of the demise of the Steller's sea cow will ideally assist in formulating research and recovery priorities for extant species of dugong and manatee where minimal emphasis has been placed on the genetic component of population viability and recovery.

Problems

In the 2012 field season we did not face any significant problems working on this project. However, there was some delay with getting funding and buying supplies for the development of the genetic samples. This led to some delay in the production of our second-year report.

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More than 95% of the Steller's sea cow ribs and their fragments were discovered on the sand and gravel beaches of Bering Island. Ribs were washed out by storms and turned up close to the grass terrace, often mixed in with fragments of wood or cetacean remains.

Some of the Steller's sea cow bones were located on the banks of small rivers and streams. The remains are easy to identify by the darker (yellowish and brownish) coloration and sometimes the presence of green algae.

This is a "harvest" of Steller's sea cow remains from only one trip along the beaches of Bering Island.

At low tide the incredible biomass of Laminaria sp. becomes visible. Along with other species of brown alga, Laminaria were the main food resources for Steller's sea cow. The total biomass of brown algae on the Commander Islands is at least a million tonnes. This picture was taken from Tolsty Cape on the east coast of Bering Island – typical Steller's sea cow habitat.

This photo shows a Steller's sea cow rib with pathological changes in the bone structure. The origin of such pathology is probably bone trauma before the animal died. Looking at the shape of damages, we suggest that an iron trident with triangle teeth struck the sea cow in its rib, and thus caused these pathological bone changes.